Linkages Between Physical Activity and Nightly Salivary Cortisol in a Pilot Study of Adolescents

The Ohio State University College of Nursing

By

Louis K. Gresham

Mentor: Jodi Ford, PhD, RN

The Ohio State University

2015

Abstract

The purpose of this study was to explore associations between the frequency of moderate/strenuous physical activity among adolescents and their averaged nightly cortisol level collected over a one week time period. Prior research has supported that physical activity can be beneficial for reducing psychosocial stress. However, studies have also found significant associations between high intensity exercise and elevated salivary cortisol levels– a physiologic stress marker. Secondary data were analyzed from a pilot study of 22 adolescents recruited from one high income and one low-income census tract located in an urban area in the Midwest. Data were collected via trained interviewers over a one-week time frame in which youth completed an in-home survey and self-collected nightly saliva samples for cortisol on nights 1-6. Two survey questions were asked related to the frequency of moderate and strenuous physical activity over the prior week (range 0-10). The frequencies were summed to create a total score of moderate/strenuous physical activity over the prior week and dichotomized to compare adolescents who engaged in moderate/strenuous physical activity 6-10 per week to those who engaged 0-5 times per week. Nightly cortisol levels were assessed via ELISA assay and the week's values were averaged and logged due to the skewed distribution. Spearman rho correlation between the total score of moderate/strenuous physical activity and the averaged nightly cortisol was modest (0.37, $p<0.09$) and the ANOVA bivariate analysis indicated adolescents who engaged in moderate/strenuous physical activity 6-10 per week had higher mean cortisol values compared to their less active peers ($p<0.05$). Longitudinal research on the effects of frequent/moderate physical activity on adolescents' cortisol levels and subsequent health outcomes is needed.

Chapter I: Introduction

Recent international recommendations for adolescent physical activity is to engage in at least 60 minutes of moderate to vigorous activity each day of the week due to the short and long-term health benefits (U.S. Department of Health and Human Services, 2008; O'Donovan, 2010; Strong et al., 2005). The U.S. Department of Health and Human Services recommends that kids aged 6-17 should participate in physical activity for at least 60 minutes each day of the week with the majority of the activity consisting of moderate to vigorous-intensity (2008). Positive changes in the immune system have been found in several studies with daily, moderate physical activity (Gleeson, 2007). Although moderate physical activity can be beneficial, studies have suggested that more intense physical activity may inhibit the typical immune response. Prolonged, strenuous physical activity can elicit a temporary decrease in immune functioning that can leave the individual susceptible to minor infections (Gleeson, 2007).

However, the volume, type, and intensity of the exercise will influence the health benefits that may be obtained (Sothern 1999). Previous studies have shown acute increases in levels of cortisol in salivary samples following exercise (VanBruggen, Hackney, McMurray, & Ondrak, 2011). Cortisol is a lipophilic steroid released continuously by the hypothalamic-pituitary-adrenal axis with increases in levels found in response to physical or psychosocial stress. However, studies have reported that varying intensities of exercise elicit different responses by the body. McGuigan's study (2004) found that there was a statistically significant increase in salivary cortisol levels immediately after strenuous exercise and that the low intensity exercise did not produce any significant change. Hill (2008) had similar results: 30 minutes of exercise at the subject's maximum oxygen intake

(VO_2 max) of 60% and 80%, moderate to high intensity exercise, caused significant, high levels of cortisol in blood samples drawn immediately after the activity. This suggests that a threshold intensity of about 60% VO_2 max would be enough to cause a significant increase in serum and salivary cortisol (Hill, 2008). Repeated, short bursts of high intensity cycling in boys aged 15-16 years old produced statistically significant elevations in salivary cortisol levels 5 minutes post exercise (Thomas et al., 2009). This reflects the natural engagement of children and adolescent in brief, high-intensity physical activity (Thomas et al., 2009). This may be seen in physical education, recess, or after-school activity. Thomas et al. (2009) support the suggestion that moderate/strenuous physical activity results in significant increases in salivary cortisol levels. In the pubertal transition years, participation in organized sports and structured activity increase (Strong et al., 2005). Improving on their gross motor skills and learning new ways to incorporate enjoyable physical activity into their everyday life sets adolescents up an active lifestyle (Strong et al., 2005). Establishing healthy lifestyle habits in childhood and adolescents are important because the risk of chronic disease begins in these ages (Warburton, 2006).

Although many studies have been done on cortisol changes in response to exercise among elite adolescent athletes, there is a lack of studies and data on how high intensity physical activity impacts the physiology and hormonal responses in the general population of adolescents (Thomas et al. 2009). This study looks to advance the knowledge of general adolescents in regards to how their participation in moderate/strenuous physical activity impacted their nightly, salivary cortisol levels. The purpose of this study was to explore associations between the frequency of moderate/strenuous physical activity among adolescents and their averaged nightly cortisol level collected over a one-week time period.

Chapter II: Methodology

Study Design

This is a secondary data analysis from a pilot study of twenty-two adolescents aged 11-17 years old. Both the pilot study and the secondary data analyses were approved by The Ohio State University Institutional Review Board. The pilot study recruited subjects from a high-income (n=12) and low-income (n=10) census tract in an urban area in the Midwest. Data were collected via trained interviewers over a one-week time frame in which youth completed an in-home survey via Computer Assisted Self-Interviewing (CASI) survey on the first day of data collection that included items about physical activity. In addition, the interviewers instructed the adolescents at the beginning of the week on how to collect the cortisol sample each night prior to bedtime for the six nights of the study period and to store the samples in the household freezer until collected by the interviewer at the end of the week. All saliva samples were collected via unstimulated passive saliva drool – a cost-effective and highly recommended method that has been used successfully with adolescents in prior research (Granger et al., 2007; Hanrahan, McCarthy, Kleiber, Lutgendorf, & Tsalikian, 2006). Adolescents were also instructed to avoid the following within 20 minutes of collection due to potential interactions with cortisol levels: eating a large meal; consumption of dairy, high sugar, acidic and caffeinated products; and teeth brushing. Specific instructions for collection include:(1) rinse mouth with water 10 minutes prior to collection, (2) allow saliva to pool in the mouth, (3) with head tilted forward, allow the saliva to flow down the straw into the cryovial, (4) repeat as needed until at least 1 ml of saliva has been collected in the cryovial, (5) record the date and time of collection on the label of the cryovial; and (6) place the tube in the household freezer until collected by the interviewer at visit 2 (on day 7). On average, 1 to 5 ml of saliva can be collected within 3 to 5 minutes using the passive drool method (Granger et al., 2007). Participants were given a $30 gift card as an incentive for their participation in the pilot study saliva collection.

Measures

Dependent variable: Averaged nightly salivary cortisol

Cortisol is an essential adrenal steroid hormone released by the hypothalamic-pituitary-adrenal (HPA) axis – a fundamental system that produces and regulates the hormones of the body's stress response. Blood sugar levels, gluconeogenesis, immune responses, anti-inflammatory actions, blood pressure, and central nervous system activation are all impacted by what is commonly known as the "stress hormone." Cortisol levels naturally fluctuate across the course of a day in a circadian rhythm. Levels peak around 0830 and decline through the course of the day, reaching the nadir around 0000 and beginning to rise again around 0200-0300 (Chan, 2010). In addition, cortisol levels typically rise in response to an acute psychological or physical stressor.

Cortisol was assayed from the saliva via Salimetrics™ high sensitivity enzyme immunoassay (EIA) cortisol kit at Microgen Laboratories in Marque, TX. Samples were stored at -80 degree Celsius after collection from the home and shipped on dry ice. To conduct the assay, the saliva sample was thawed completely, vortexed and centrifuged at 1500 x g (@3000 rpm) for 15 minutes. All samples from each subject were run at the same time and each sample was analyzed in duplicate; the inter-assay and intra-assay coefficients of variation were less than 5% and 9% respectively. The cortisol values were logged for this study due to positive skew and the mean logged cortisol level over the week was used for analyses.

Independent Variables: Physical Activity

On day 1 of the study, two survey questions were asked related to the frequency of moderate and strenuous physical activity over the prior week (range 0-10). The first question read: "During a typical week (7 days), how many times on average do you do the following kinds of activities for more than 15 minutes during your free time? Strenuous exercise where your heart beats rapidly such as running, jogging, basketball, cheerleading, vigorous cycling, rollerblading, soccer, martial arts, aerobics, etc." The participants had the choice of these responses: 0 times per week, 1 time per week, 2 or 3 times per week, 4 or 5 times per week, 6 or 7 times per week, and 8 or more times. The second question read: "Moderate exercise (exercise that is not exhausting), such as fast walking, easy bicycling, volleyball, easy swimming, etc." The participants had the same choice of responses as above.

The frequencies were summed to create a total score (range 0-10) of moderate/strenuous physical activity over the prior week. In addition, the scores were dichotomized to compare adolescents who engaged in moderate/strenuous physical activity 6-10 times per week to those who engaged 0-5 times per week.

Analytic Strategy

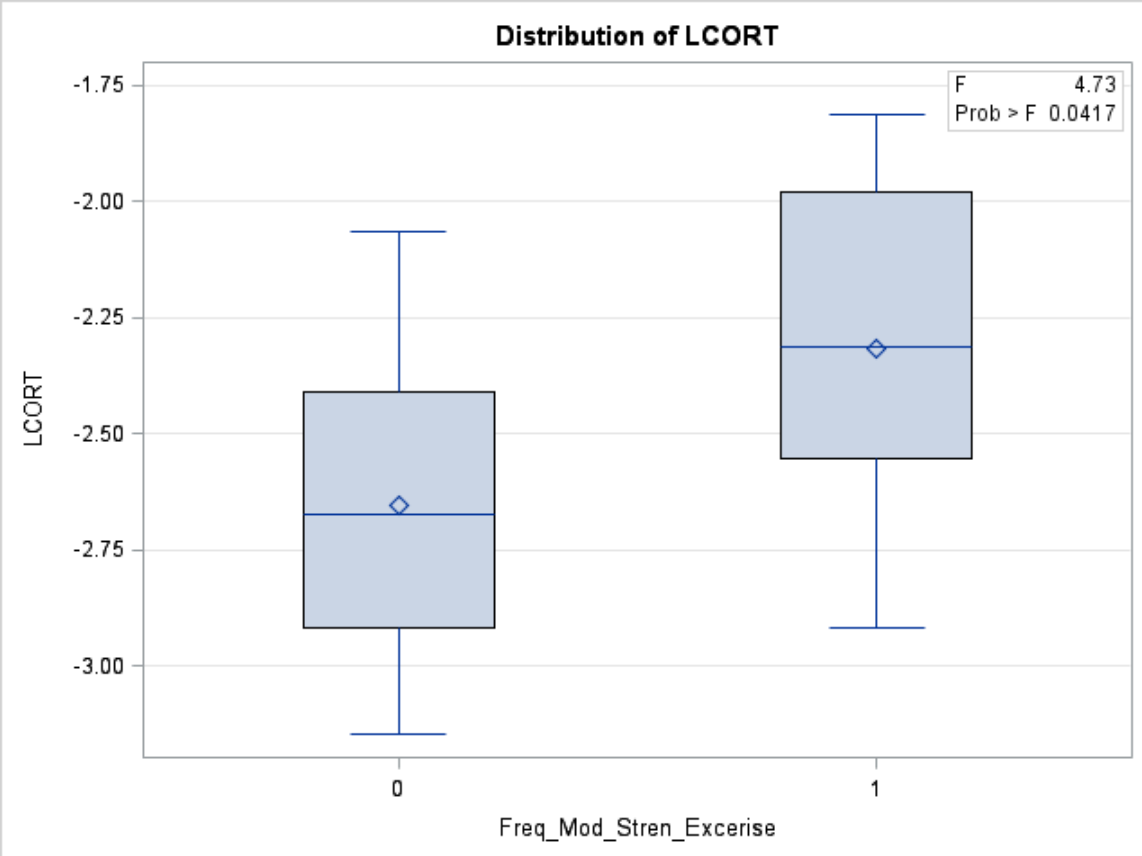
Descriptive analyses were conducted to describe the study sample. Bivariable analyses were then conducted to examine (1) the associations between the total score of moderate/strenuous physical activity over the week and the averaged nightly salivary cortisol level via Spearman rho correlation and (2) the differences in the averaged nightly cortisol levels between those youth who engaged in moderate/vigorous physical activity at least 6-10 times per week versus 0-5 times per week via ANOVA analyses with a between-subjects design. A one-way analysis of variance is useful when the analysis has a single predictor variable that can assume two values (moderate/strenuous exercise as a yes or no) and a dependent variable, salivary cortisol levels, that is measured on a ratio scale (O'Rourke et al, 2005). In this type of analysis, there is only one independent variable. In this study, the independent variable was the participation in moderate/strenuous physical activity or not. The between-subjects design means that each participant was only in one of the two groups and that the comparison was made between the two groups of participants. The average salivary cortisol levels of the two groups were compared to see if there was a statistically significant difference between the active group and their less active peers.

Chapter III: Results

The participants included a total of 22 adolescents aged 11-17 from one high-income census tract (n=12) and one low-income census tract (n=10) in an urban area in the Midwest. There were an equal number of male and females in the study. The average age of the sample was 13.6 years. The mean of the total score of moderate/strenuous physical activity was 6.0 with a range of 1-10. The group of more active adolescents, who engaged in moderate/strenuous physical activity 6-10 times per week, was comprised of 9 subjects (40.9% of the participants). The less active group, who engaged in moderate/strenuous physical activity only 0-5 times per week, had 13 adolescents. The mean nightly salivary cortisol level (before logged) was 0.08ug/dL with a range 0.04-0.163.

The results of the Spearman rho correlation between the total score of moderate/strenuous physical activity and the averaged nightly cortisol was modest ($r=0.37$, $p<0.09$). Although this correlation was not significant, it warrants further research.

The dichotomized groups were analyzed via a one-way ANOVA bivariate analysis. This analysis indicated that adolescents who engaged in moderate/strenuous physical activity 6-10 times per week had higher mean cortisol values compared to their less active peers ($F=4.73$, $p<0.05$). The following figure illustrates the differences in the logged means between the two groups.



This box and whisker plot was made from the data of the averaged, nightly salivary cortisol level from each of the participants from the two respective dichotomized groups. The box and whisker plot on the left (Freq_Mod_Stren_Exercise = 0) is the data from the group that participated in moderate/strenuous physical activity 0-5 times per week. The box and whisker plot on the right (Freq_Mod_Stren_Exercise = 1) is the data from the group that participated in moderate/strenuous physical activity 6-10 times per week. The diamonds represent the average of the two groups while the horizontal line in each shaded box marks the median value of the two groups. The shaded boxes represent the interquartile range. The horizontal lines that define the edges of the boxes represent the median of the first and third quartiles. The horizontal lines at the end of the whiskers on each box represent the minimum and maximum values for the two data sets.

Chapter IV: Discussion

In summary, this study suggests that the more frequent participation in moderate/strenuous physical activity elicits, on average, a higher level of cortisol in salivary samples at bedtime in adolescents aged 11-17. The ANOVA bivariate analysis of the dichotomized groups found this result to be statistically significant ($p<0.05$). This suggestion supports previous studies that have found moderate/strenuous physical activity to increase circulating cortisol levels (Hill, 2008; McGuigan, 2004). Physical activity has been previously found to have health benefits for the body (Sothern, 1998; Strong et al., 2005; Warburton, 2006). However, prolonged, strenuous physical activity can leave the body vulnerable to infection (Nieman, 2003). Further longitudinal research is needed to explore the impact of physical activity on cortisol levels over the transition from adolescence to young adulthood.

The salivary samples in this study were collected each evening for nights 1-6. The time between completion of physical activity for the day and the collection of the salivary sample may have allowed cortisol levels to decrease or return to normal, which only takes a few hours according to Urhausen (1995). Because the pilot study did not collect data on timing of physical activity with respect to the cortisol collection, it is difficult to discern if the cortisol was elevated due to the normal increase after physical activity or if the increase persisted and did not decline among those who engaged in more moderate/vigorous activity. In order to capture the peak in cortisol after physical activity, multiple saliva samples could be taken before and after exercise. Immediate collection of salivary samples upon termination of exercise, however, may be too early to test for a bodily response (Thomas et al., 2009; Woods, 1999). Woods (1999) suggests that immediate collection of samples post-exercise may be a reason for conflicting results from various studies. Thomas et al. (2009) further explain that cortisol production would not be apparent until several minutes after exercise. Other researchers agree; suggested deferral of salivary collection should be a half hour (Kirschbaum & Hellhammer, 1989). However, the ANOVA bivariate analysis of the dichotomized groups found that the more active adolescents, moderate/strenuous physical activity 6-10 times per week, had higher mean salivary cortisol levels, on average, than their less active peers over the 6-day time period. An important implication may be that adolescents who participate in moderate/strenuous physical activity 6-10 times or more per week may have cortisol levels that do not return to baseline as quickly as previously thought. In other words, moderate/strenuous physical activity may have a longer impact on adolescents' cortisol levels than previously thought. It would be important to see if these cortisol levels return to baseline because chronically elevated cortisol levels may impact adolescents' growth, development, and long-term health.

Despite the significant findings, there were limitations of the study that may have impacted the findings and interpretation of the data. Since this was a pilot study that was not designed to address the specifics of physical activity related to salivary cortisol levels, limitations were encountered. First, the sample size was limited due to the nature of a pilot study and 22 of the original 26 individuals' data were usable. Four of the students' data was discarded because they either did not adhere to the protocol for the saliva sample collection or data was missing on physical activity measures from the survey. The total number of participants was thus 22 instead of 26. Second, salivary samples were collected one time each day. The participants provided samples in the evening before bedtime in this study. This was done to help increase adherence to the collection protocol. Cortisol follows a diurnal curve and collecting samples to plot an individual's baseline would be beneficial for the analysis. Recommended times of minimal collection for plotting their diurnal curve would involve sampling immediately upon waking, 30 minutes after waking in order to capture the peak, and at bedtime (Adam & Kumari, 2009). However, knowing that consistency and adherence to protocol was critical to being able to use a participant's data in this study, only one sample was collected per day.. Consistent adherence to protocol and collection of samples on six consistent days were methods that were implemented in this study in order to increase the reliability of the data (Hellhammer et al., 2007). Third, because the study was not focused solely on physical activity, the measures were limited to self-report rather than more objective measures such as actigraphy.

Despite these limitations, the findings suggest avenues for future research. For example, the full study has entered into the field period and includes measures on youth's daily physical activity that could be compared to their nightly cortisol levels. An objective method of measuring a participant's physical activity, rather than subjective self-report, would be beneficial for accuracy of the data. Had the pilot study been able to compare the participants' daily physical activity to the respective night's cortisol sample, the analysis may have been able to suggest how the daily physical activity impacted their nightly salivary cortisol levels. Overall, this may suggest a difference between nightly cortisol levels depending on the amount and intensity of physical activity for the respective day. In addition, the full study is collecting hair for cortisol – a novel, yet valid measure of chronic stress as each 1 cm of hair growth approximates the mean cortisol levels of the corresponding month. (Russell, Koren, Rieder, & Van Uum, 2012). This is beneficial for longitudinal study because it allows researchers to analyze participants' cortisol levels from previous months. In contrast, salivary samples for cortisol are reflective of acute increases in cortisol (Sauvé, Koren, Walsh, Tokmakejian, & Van Uum, 2007). Measuring cortisol in hair samples is beneficial because the collection process is non-invasive, the participant's cortisol levels are not affected by the collection process, the hair samples can be stored at room temperature and is stable for years, and only free cortisol is measured as it is not affected by cortisol binding globulin (Russell, Koren, Rieder, & Van Uum, 2012). Future studies that utilizes salivary and hair cortisol samples would be beneficial as acute and previous month's cortisol levels could be analyzed for the participants.

Works Cited

Adam EK, Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology. 2009;34(10):1423-36.

Chan, S. (2010). Replication of cortisol circadian rhythm: New advances in hydrocortisone replacement therapy. *Therapeutic Advances in Endocrinology and Metabolism, 1*(3), 129-38.

Gleeson, M. (2007). Immune function in sport and exercise. *Journal of Applied Physiology (1985), 103*(2), 693-9.

Granger DA, Kivlighan KT, Fortunato C, Harmon AG, Hibel LC, Schwartz EB, Whembolua GL. Integration of salivary biomarkers into developmental and behaviorally-oriented research: problems and solutions for collecting specimens. Physiology & Behavior. 2007;92:583-90.

Hanrahan K, McCarthy AM, Kleiber C, Lutgendorf S, Tsalikian E. Strategies for salivary cortisol collection and analysis in research with children. Applied Nursing Research. 2006; 19: 95-101.

Hellhammer J, Fries E, Schweisthal OW, Schlotz W, Stone AA, Hagemann D. Several daily measurements are necessary to reliably assess the cortisol rise after awakening: state- and trait components. Psychoneuroendocrinology. 2007;32(1):80-6.

Hill, E. (2008). Exercise and circulating cortisol levels: The intensity threshold effect. *Journal of Endocrinological Investigation, 31*(7), 587-591.

Kirschbaum, Clemens, Hellhammer,Dirk H.,. (1989). Salivary cortisol in psychobiological research: An overview. *Neuropsychobiology, 22*(3), 150-169.

McGuigan, M. (2004). Salivary cortisol responses and perceived exertion during high intensity and low intensity bouts of resistance exercise. *Journal of Sports Science & Medicine, 3*(1), 8-15.

Nieman, D. C. (1999). Exercise and immune function: Recent developments. *Sports Medicine, 27*(2), 73.

O'Donovan, G. (2010). The ABC of physical activity for health: A consensus statement from the british association of sport and exercise sciences. *Journal of Sports Sciences, 28*(6), 573-91.

O'Rourke, N., Hatcher, L., Stepanski, E. J., (2005). *A step-by-step approach to using SAS for univariate & multivariate statistics.* New York: Wiley-Interscience.

Russell E, Koren G, Rieder M, Van Uum S. Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions. Psychoneuroendocrinology. 2012;37(5):589-601.

Sauvé B, Koren G, Walsh G, Tokmakejian S,Van Uum SH,. (2007). Measurement of cortisol in human hair as a biomarker of systemic exposure. *Clinical and Investigative Medicine.Médecine Clinique Et Experimentale, 30*(5), 183-91.

Sothern, M. (1999). The health benefits of physical activity in children and adolescents: Implications for chronic disease prevention. *European Journal of Pediatrics, 158*(4), 271-4.

Strong WB, Malina RM, Blimkie CJ, Daniels SR, Dishman RK, Gutin B, Hergenroeder AC, Must A, Nixon PA, Pivarnik JM, Rowland T, Trost S,Trudeau F,. (2005). Evidence based physical activity for school-age youth. *The Journal of Pediatrics, 146*(6), 732-7.

Thomas, N. E., Leyshon, A., Hughes, M. G., Davies, B., Graham, M., & Baker, J. S. (2009). The effect of anaerobic exercise on salivary cortisol, testosterone and immunoglobulin (A) in boys aged 15-16 years. *European Journal of Applied Physiology, 107*(4), 455-461.

Urhausen, A. (1995). Blood hormones as markers of training stress and overtraining. *Sports Medicine, 20*(4), 251-276.

U.S. Department of Health and Human Services. *Physical Activity Guidelines for Americans*. Washington, DC: U.S. Department of Health and Human Services; 2008.

VanBruggen, M. D., Hackney, ,Anthony C., McMurray, R. G., & Ondrak, K. S. (2011). The relationship between serum and salivary cortisol levels in response to different intensities of exercise. *International Journal of Sports Physiology & Performance, 6*(3), 396-407.

Warburton, D. (2006). Health benefits of physical activity: The evidence. *CMAJ.Canadian Medical Association Journal, 174*(6), 801-9.

Woods, J. (1999). Exercise and cellular innate immune function. *Medicine and Science in Sports and Exercise, 31*(1), 57-66